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Heat tolerance and expression of protein synthesis elongation factors, EF-Tu and EF-1α, in spring wheat

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Abstract. Protein elongation factors, EF-Tu and EF-1α, have been implicated in cell response to heat stress. We investigated the expression (accumulation) of EF-Tu and EF-1α in mature plants of spring wheat cultivars Kukri and Excalibur, and tested the hypothesis that cultivars with contrasting tolerance to heat stress differ in the accumulation of these elongation factors under prolonged exposure to high temperature (16 days at 36/30°C). In addition, we investigated the expression of EF-Tu and EF-1α in young plants experiencing a 24-h heat shock (43°C). Excalibur showed better tolerance to heat stress than Kukri. Heat stress induced accumulation of EF-Tu and EF-1α in mature plants of both cultivars, but to a greater extent in Excalibur. Young plants did not show appreciable accumulation of EF-Tu in response to heat shock. However, these plants showed increased accumulation of EF-1α and the accumulation appeared greater in Excalibur than in Kukri. The results support the hypothesis that EF-Tu plays a role in heat tolerance in spring wheat. The results also suggest that EF-1α may be of importance to wheat response to heat stress.

Introduction

Exposure to high temperature or heat is a common stress for wheat (*Triticum aestivum* L.), restricting its growth and productivity (Boyer 1982; Lobell and Asner 2003; Wahid *et al*. 2007), and reducing the quality of harvested products (Stone and Nicolas 1995). The heat-induced reduction in wheat yields and quality is attributed to negative effects of heat stress on plant metabolic and physiological processes (Wahid *et al*. 2007). Heat stress also causes denaturation and aggregation of many proteins, and injury to cellular membranes (Levitt 1980; Larkindale *et al*. 2005).

Plants including wheat are adapted to diverse environments and have mechanisms that enable them to resist (avoid or tolerate) the negative effects of heat stress (Levitt 1980). Of all mechanisms of heat resistance, protein thermal stability (Levitt 1980) and heat shock proteins (Vierling 1991; Feder and Hofmann 1999) are of crucial importance. Protein thermal stability constitutes the basis for plant thermotolerance (Levitt 1980), and heat shock proteins (HSPs) play a central role in heat tolerance by acting as molecular chaperones; that is, they bind and stabilise heat-labile proteins, protecting them from thermal aggregation (Vierling 1991; Hendrick and Hartl 1993; Feder and Hofmann 1999; Lee and Vierling 2000; Basha *et al*. 2004).

Some proteins other than HSPs play a role in heat tolerance by acting as molecular chaperones. Examples include the prokaryotic protein synthesis initiation factor IF2, protein synthesis elongation factors EF-G (Caldas *et al*. 2000) and EF-Tu (Caldas *et al*. 1998; Malki *et al*. 2002) and the mammalian mitochondrial translation elongation factor EF-Tu-mt (Suzuki *et al*. 2007). These proteins perform a chaperone function by interacting with unfolded proteins, thereby protecting them from thermal aggregation.

A recent study suggested that chloroplast protein synthesis elongation factor, EF-Tu, may contribute to heat tolerance in spring wheat (Ristic *et al*. 2007*a*). The native precursor of this protein, purified from spring wheat, displays chaperone activity and reduces thermal aggregation of photosynthetic enzyme Rubisco activase in a concentration-dependent manner (Ristic *et al*. 2007*a*). If EF-Tu plays a role in heat tolerance, it is reasonable to expect that this protein may be upregulated during exposure to heat stress. One of the objectives of this study was to investigate this possibility. We examined heat tolerance and the expression (relative amount/accumulation) of chloroplast EF-Tu in two cultivars of spring wheat.

Several studies suggest that the cytosolic counterpart of chloroplast EF-Tu, EF-1α, may also play a role in heat tolerance. This protein is upregulated in *Gillichthys mirabilis* (Cooper) during heat stress (Buckley *et al*. 2006), and in mammalian cells, EF-1α mediates cell response to high temperature by activating the heat-shock transcription factor 1, thereby regulating the expression of heat shock proteins (Shamovsky *et al*. 2006). In addition, mammalian EF-1α displays chaperone-like activity as it interacts with unfolded or partially folded proteins (Hotokezaka *et al*. 2002). EF-1α is highly conserved (Riis *et al*. 1990; Bunai *et al*. 2006), and it is possible that this protein may be of importance to heat tolerance in plants including wheat. As a step in testing this possibility, we examined the expression of EF-1α in two cultivars of spring wheat.

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Materials and methods

Plant material

Heat tolerance and expression of two protein synthesis elongation factors, chloroplast EF-Tu and cytosolic EF-1α, were investigated in two Australian cultivars of spring wheat (*Triticum aestivum* L.), Kukri and Excalibur. These cultivars were chosen because they exhibit contrasting tolerance to drought stress, with Excalibur being described as drought-tolerant and Kukri as drought-susceptible (Izanloo *et al*. 2008). In addition, our preliminary study suggested that Kukri and Excalibur differ in heat tolerance as under prolonged exposure to high temperature (14 days at 36/30°C, day/night) Excalibur displayed better ability to delay damage to thylakoid membranes and retain chlorophyll than Kukri (Fig. 1*A*, *B*).

Seeds of the cultivars were obtained from the Australian Centre for Plant Functional Genomics, The University of Adelaide, SA. Two experiments were conducted under controlled environmental conditions. In the first experiment, we investigated heat tolerance and the expression of EF-Tu and EF-1α in mature plants (at flowering stage) experiencing a 16-day period of heat stress. We chose mature plants because under field conditions wheat is more likely to encounter prolonged exposure to elevated temperature at flowering stage. In the second experiment, we investigated the expression of EF-Tu and EF-1α in young (36-day-old) plants after exposure to a short-term heat shock (24 h, 43°C).

Experiment with mature plants

Seeds of each cultivar were sown in 10 pots (five seeds per pot; pot diameter at the top and the bottom was 21 and 16 cm, respectively; pot height was 20 cm) containing potting soil Metro Mix 200 (Hummert International, Topeka, KS, USA). Plants were grown in a growth chamber [Conviron, PGW-36 (Winnipeg, MB, Canada); day/night temperature, 22/17°C; relative humidity (RH), 70%; photoperiod, 16 h; PPF, 280 µmol m^{-2} s^{-1} (Sylvania cool white fluorescent lamps)], watered daily, and fertilised weekly using 'Miracle Gro' fertiliser (Stern's Miracle-Gro Products Inc., Port Washington, NY, USA) according to manufacturer instructions. At flowering stage [growth stage Feekes 10.5.1 (Large 1954)], plants of each cultivar were divided into control (five pots) and treatment/heat-stress (five pots) groups. Five plants in the control group and 10 plants in the treatment group were then randomly chosen (one plant per pot in the control group and two plants per pot in the treatment group), and one flag leaf per selected plant was tagged (total of five flag leaves in the control group and 10 flag leaves in the treatment group were tagged). The tagged leaves were later used for assessment of heat tolerance. The control group was maintained under the initial growth conditions (described above), and the treatment group was exposed to heat stress for 16 days [day/night temperature of 36/30°C; RH, 90–100%; photoperiod, 16 h; PPF, 280 µmol m^{-2} s^{-1} (Sylvania cool white fluorescent lamps)] in a growth chamber [Conviron (PGW-36)]. The heat treatment started by gradually increasing the temperature from 22 to 36°C over 1 h. To minimize/avoid possible dehydration of the leaf tissue during heat treatment, pots of the treatment and control group were kept in trays containing water ~1 cm deep. Following stress treatment, plants of both control and treatment groups were transferred to the greenhouse and allowed to recover at ambient temperature until

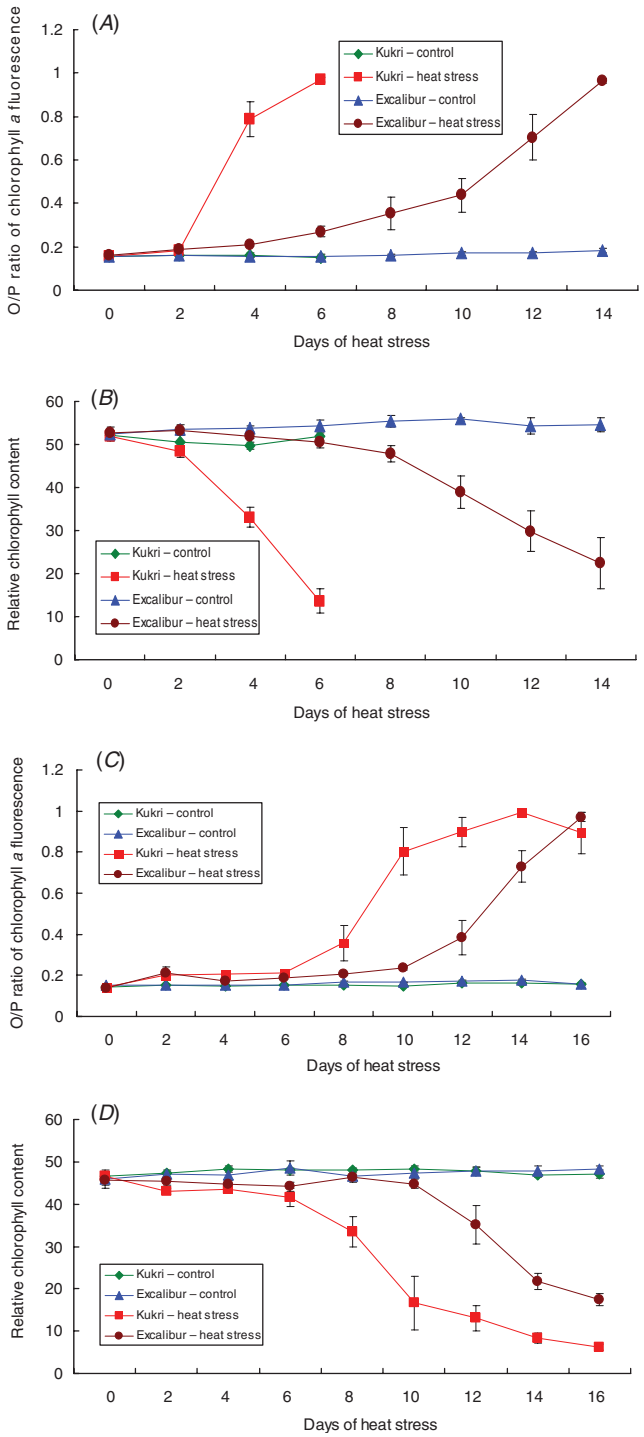

Fig. 1. Spring wheat cultivar Excalibur shows greater delay in injury to thylakoid membranes (*A*, *C*) and lower loss of chlorophyll (*B*, *D*) under heat stress conditions than cultivar Kukri. (*A*, *B*), data from the preliminary experiment; mature plants (flowering stage) were exposed to 36/30°C (day/night; RH, 90–100%) in a growth chamber for 14 days. (*C*, *D*), mature plants (flowering stage) were exposed to 36/30°C (day/night) for 16 days. Increases in the ratio of constant fluorescence and the peak of variable fluorescence (O/P) indicate injury to thylakoids (Krause and Weis 1984). Chlorophyll *a* fluorescence and chlorophyll content were measured on the same flag leaves at indicated days of stress treatment. Bars indicate s.e.: control plants, $n = 5$; heat stressed plants, $n = 10$.

harvest maturity. In the greenhouse, air temperature was measured at hourly intervals (average daily temperature in the greenhouse was 22.7 ± 3.0°C during the period of experimentation). Relative levels of EF-Tu and EF-1α were determined after 7 days of heat stress, and plant heat tolerance was assessed after 0, 2, 4, 6, 8, 10, 12, 14, and 16 days of stress treatment and at harvest maturity. For EF-Tu and EF1α analysis, samples of leaf tissue were obtained from the flag leaf blades from three randomly selected plants (each plant was taken from a different replicate pot) from both control and heat-stressed groups. The same flag leaf blades were also used to obtain samples for assessment of thermal aggregation of total soluble leaf proteins (see below). Collected leaves were immediately frozen in liquid nitrogen and stored at −80°C until further use.

Experiment with young plants

The experimental design was similar to that for mature plants with some modifications. Seeds of each cultivar were sown in six pots (five seeds per pot) containing Metro Mix 200 potting soil. Plants were grown in a growth chamber [Conviron (PGW-36)] under growth conditions similar to those for mature plants. Plants were watered and fertilised as described above. Thirty-six-day-old plants of each cultivar were divided into control (three pots) and treatment groups (three pots). The control group was maintained under the same conditions (22/17°C, day/night), and the treatment group was exposed to 43°C (RH, 90–100%) for 24 h in a growth chamber (Gallie *et al*. 1997). The temperature was gradually increased from 22 to 43°C over 1 h. Exposure time for heat shock treatment started when the temperature reached 43°C. For EF-Tu and EF-1α analysis, leaf tissue was collected from both control and heat-shocked plants immediately after heat treatment. The youngest fully expanded leaves were collected from three randomly selected plants from each group (one plant from each pot). Collected leaves were immediately frozen in liquid nitrogen and stored at −80°C until further use.

Assessment of heat tolerance

Heat tolerance was assessed by examining the stability of thylakoid membranes (Krause and Weis 1984; Ristic *et al*. 2008*a*), measuring chlorophyll content (Reynolds *et al*. 1998) and the thermal (heat-induced) aggregation of leaf protein extracts (Fu *et al*. 2008) and by assessing plant yield traits (number of heads and kernels per plant, number of kernels per head, single kernel mass, and total kernel mass per plant) at harvest maturity (Prasad *et al*. 2008). The stability of thylakoid membranes was assessed by measuring chlorophyll *a* fluorescence and determining the ratio of constant fluorescence (O) and the peak of variable fluorescence (P) (Ristic *et al*. 2007*b*). Fluorescence was measured at room temperature using a pulse modulated fluorometer (Model OS5-FL, Opti-Sciences, Hudson, NH, USA). Chlorophyll content was measured on the same flag leaves, in the same blade area that was used for fluorescence measurements, by using a self-calibrating chlorophyll meter (SPAD meter, Model 502, Spectrum Technologies, Plainfield, IL, USA) (Ristic *et al*. 2007*b*). Five flag leaves from the control group and 10 flag leaves from the treatment group of each cultivar were used for measurements. At maturity, all plants except those used for protein analysis (EF-Tu, EF-1α, and protein thermal aggregation) were harvested and analysed for yield traits (Prasad *et al*. 2008).

Thermal aggregation of leaf protein extracts

The thermal aggregation of leaf protein extracts was assessed using light scattering (Fu *et al*. 2008). Total soluble proteins were extracted from flag leaves collected from three control and three heat-stressed plants (proteins from each control and each heat-stressed plant were extracted and used for light scattering separately). Proteins were extracted in an extraction buffer [50 mM Tris–HCl (pH 8.0), 2 mM EDTA, 10% glycerol and 1% protease inhibitor cocktail (v/v, Sigma, St Louis, MO, USA)], and protein concentration was determined by using the *RC DC* Protein Assay (BioRad, Hercules, CA, USA). Protein aliquots (200 µL; protein concentration 300 µg mL^{-1}) were incubated at 53°C for 45 min in a temperature-controlled micro-multi cell spectrophotometer (Shimadzu, Tokyo, Japan), and the thermal aggregation of proteins was assessed by monitoring light scattering at 320 nm during incubation.

EF-Tu and EF-1α analysis

Chloroplast EF-Tu and cytosolic EF-1α were analysed by 1-D SDS–PAGE and immunoblotting (Ristic *et al*. 2008*a*). Total soluble proteins were extracted from the leaf tissue, and protein content was determined using the *RC DC* Protein Assay (BioRad). Extracted proteins were separated on 10% polyacrylamide gels. Equal amounts of protein (15 µg per well) were loaded on the gels. Following electrophoresis, proteins were transferred to a PVDF membrane (BioRad). Blots were probed for EF-Tu by using a maize anti-EF-Tu polyclonal antibody (Bhadula *et al*. 2001). For EF-1α analysis, an antibody was raised using a synthetic peptide of amino acids (CDQINEPKRPSEKP) deduced from the nucleotide sequence of barley EF-1α cDNA (Sutton and Kenefick 1994; GenBank Accession Number: L11740). The oligopeptide was conjugated with keyhole limpet hemacyanin and used for the production of antiserum in rabbits (Sigma Genosys Biotechnologies, TX, USA). The antibody was then purified by affinity column by Sigma Genosys Biotechnologies. Anti-EF-Tu and anti-EF-1α blots were developed using a chemiluminescent ECL western blotting detection kit (GE Healthcare, Buckinghamshire, UK).

Statistical analysis

Data on heat tolerance were analysed by using PROC TTEST procedures in SAS (SAS Institute, Cary, NC, USA). The data on chlorophyll *a* fluorescence, chlorophyll content and growth and yield traits had five replications (five different plants in five different pots) for the control treatment and 10 (10 different plants in five different pots, two per pot) for heat stress treatment for each cultivar. For data on chlorophyll *a* fluorescence and chlorophyll content, effects of heat stress at each day and over different days were tested separately. All the data were analysed using a two sample *t*-test assuming unequal or equal variances after testing for variance using *F*-test.

Results

Heat tolerance

Heat stability of thylakoid membranes and leaf chlorophyll content

Exposure to high-temperature for 16 days caused damage to photosynthetic membranes (thylakoids) in the flag leaf tissue in

wheat cultivars Kukri and Excalibur as indicated by increases in the ratio of constant fluorescence (O) to the peak of variable fluorescence (P) (Krause and Weis 1984; Ristic *et al*. 2008*a*) (Fig. 1*C*). The photosynthetic membranes of the two cultivars, however, were not equally affected by heat. During the first 6 days of heat treatment, thylakoid membranes of Kukri appeared unaffected. Starting at day 8, the thylakoids of Kukri showed signs of injury, and the injury worsened as the stress treatment progressed. By comparison, in Excalibur the first sign of thylakoid injury was noticed on day 10 of heat stress. On days 12 and 14 of stress treatment, the heat injury of thylakoids increased in Excalibur, but this injury was substantially less than in Kukri. On day 16, the extent of heat injury to thylakoid membranes in Excalibur was similar to that in Kukri.

Heat stress also affected chlorophyll content in Kukri and Excalibur causing loss of this pigment. The loss of chlorophyll as a function of time was, however, different in the two cultivars (Fig. 1*D*). Kukri showed the first sign of chlorophyll loss after 8 days of heat stress, after which the chlorophyll content continued to decline reaching its minimum on day 16. In contrast, Excalibur showed the first sign of chlorophyll loss on day 12 of heat stress. After day 12, Excalibur continued to lose chlorophyll, but the chlorophyll content of this cultivar was higher than that of Kukri (Fig. 1*D*).

Thermal aggregation of total leaf proteins

When heated at 53°C, total proteins from the flag leaf tissue of control and heat-stressed plants of both cultivars formed insoluble aggregates that could be detected by an increase in light scattering. When the thermal aggregation (light scattering) of protein extracts from heat-stressed plants was expressed as a percentage of the thermal aggregation of protein extracts from control plants, a significant difference between the two cultivars was noted (Fig. 2). In Kukri, thermal aggregation of protein extracts from heat-stressed plants was not different from that in control plants. In Excalibur, however, thermal aggregation of protein extracts from heat-stressed plants was reduced compared with thermal aggregation of protein extracts from control plants (Fig. 2).

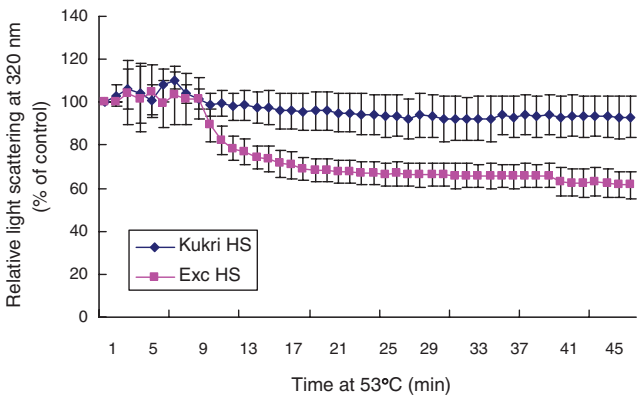

Fig. 2. Thermal aggregation of total leaf proteins from heat-stressed [7 days at 36/30°C (day/night)] mature plants of spring wheat cultivars Kukri and Excalibur (Exc). Protein thermal aggregation was assessed using light scattering (Fu *et al.* 2008). Protein aliquots were incubated at 53°C for 45 min and light scattering was monitored at 320 nm during incubation. Data represent averages of three independent measurements. Bars indicate s.e.; HS, heat stress.

Yield traits

Kukri and Excalibur showed significant differences in yield traits under both control and heat-stress conditions (Fig. 3*A–E*). Under control conditions, Excalibur produced more heads (Fig. 3*A*) and more kernels (Fig. 3*C*) per plant than Kukri. Also, the single kernel mass (Fig. 3*D*) and total mass of kernels (Fig. 3*E*) per plant was higher in Excalibur than in Kukri. Likewise, under heat stress conditions Excalibur had better yield than Kukri as heat-stressed plants of Excalibur had a higher number of heads per plant, number of kernels per head and per plant, mass of individual kernels and mass of total kernels per plant than heat-stressed plants of Kukri (Fig. 3*A–E*). When the yield traits in heat-stressed plants were expressed as a percentage of the yield traits in control plants, a significant difference between the two cultivars was noted (Fig. 3*F*). Yield of Excalibur was less affected by heat stress than yield of Kukri as indicated by number of kernels per head, single kernel mass and total kernel mass per plant (Fig. 3*F*).

EF-Tu and EF-1α expression in mature plants

Immunoblot analysis of protein extracts from the leaf tissue of mature plants revealed differences between the two cultivars in the accumulation of protein synthesis elongation factors EF-Tu and EF-1α (Fig. 4*A*). Under control conditions, the relative level of EF-Tu was higher in Excalibur than in Kukri. The relative level of EF-1α, however, appeared similar in the two cultivars under non-stress conditions. Exposure to heat stress increased the expression of EF-Tu and EF-1α in both cultivars, but the expression was substantially greater in Excalibur than in Kukri. After 7 days of heat stress, plants of Excalibur had a higher level of EF-Tu and EF-1α than plants of Kukri.

EF-Tu and EF-1α expression in young plants

The expression of EF-Tu and EF-1α in young plants was somewhat different from that in mature plants (Fig. 4*B*). Under non-stress conditions, the relative level of EF-Tu appeared similar in the two cultivars, but the relative level of EF-1α was higher in Excalibur than in Kukri (Fig. 4*B*). Exposure to heat shock (24 h at 43°C) had different effects on the expression of EF-Tu and EF-1α in the two cultivars. In Kukri, no change in the relative amount of EF-Tu was observed, and in Excalibur, a slight increase in the relative level of this protein was seen. The expression of EF-1α though, was elevated in both cultivars under heat shock conditions, but the relative amount of this protein appeared higher in heat-shocked plants of Excalibur than in heat-shocked plants of Kukri.

Discussion

Thylakoid membranes are the most heat-labile cell structures and assessment of their integrity is often used as an indicator of the plant's ability to tolerate heat stress (Santarius 1974; Schreiber and Berry 1977; Kobza and Edwards 1987; Ristic *et al*. 2008*a*). The integrity of thylakoid membranes can be assessed by measuring chlorophyll *a* fluorescence and determining the

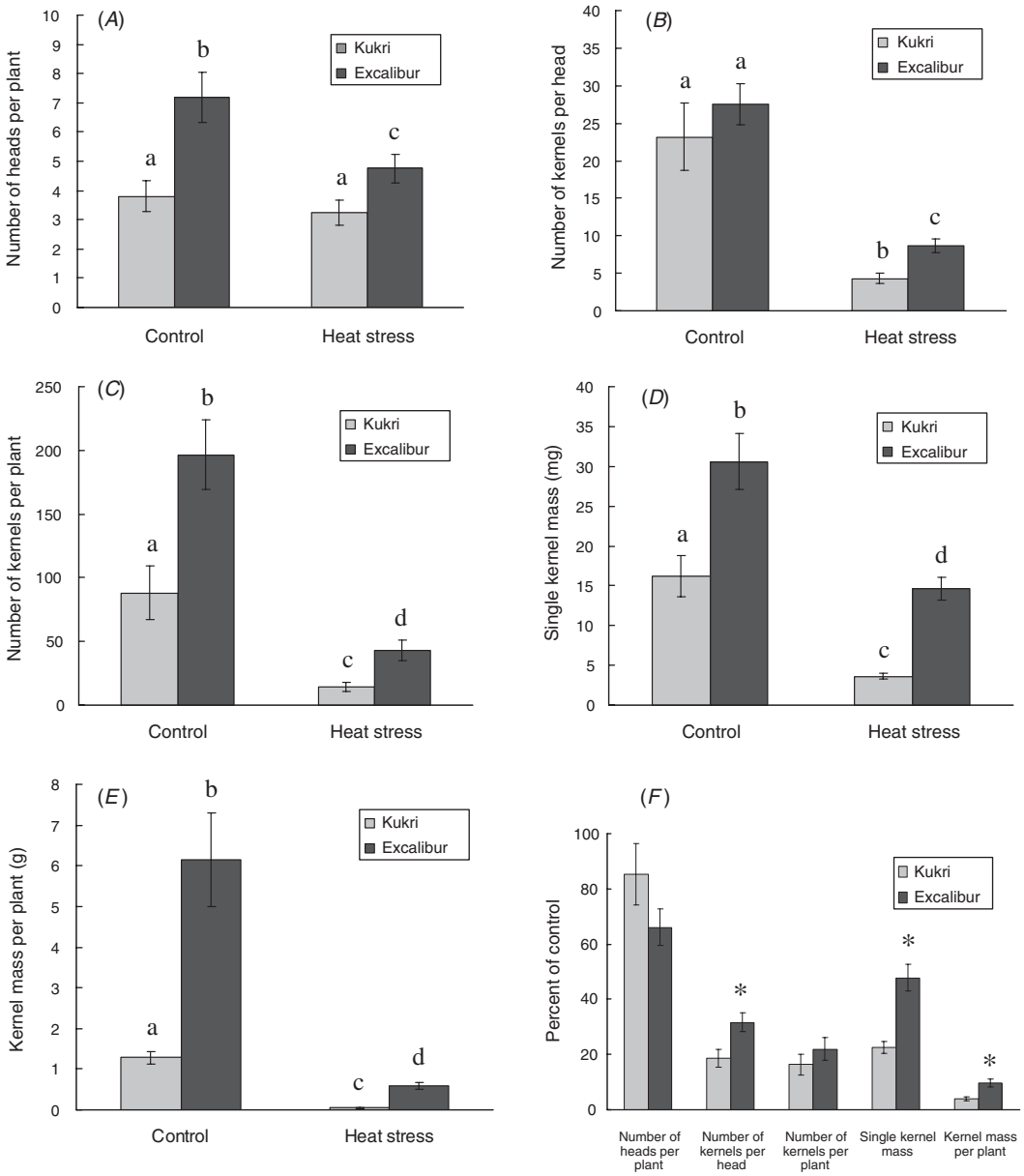

Fig. 3. Yield traits (*A–E*) and effect of heat stress on yield traits (*F*) in spring wheat cultivars Kukri and Excalibur. (*A*), number of heads per plant; (*B*), number of kernels per head; (*C*), number of kernels per plant; (*D*), single kernel mass; (*E*), kernel mass per plant. Mature plants (flowering stage) were exposed to heat stress [36/30°C (day/night] for 16 days in a growth chamber. Following heat stress, plants were allowed to recover at ambient temperature in the greenhouse until harvest maturity. Data on yield traits were obtained from 22 control and 22 heat-stressed plants. Bars indicate s.e. (*A–E*) data were averaged and used for statistical analysis; data bars assigned different letters are significantly different ($P \leq 0.05$). (*F*), data from heat-stressed plants were expressed as a percentage of control. * above the bars indicates significant difference ($P \leq 0.05$) between cultivars.

ratio of constant fluorescence (O) and the peak of variable fluorescence (P); increase in the O/P ratio indicates loss of thylakoids integrity (damage); the higher the increase the greater the damage (Krause and Weis 1984; Ristic *et al*. 2004, 2008*a*). Furthermore, chlorophyll loss can be used as an indicator of plant tolerance to heat stress (Ristic *et al*. 2007*b*). Chlorophyll loss and thermal damage to thylakoid membranes are closely associated, and measurements of chlorophyll content with a chlorophyll meter can be used to assess plant tolerance to heat stress (Ristic *et al*. 2007*b*, 2008*b*). In addition, the degree of thermal aggregation of cellular proteins (Ristic *et al*. 2004; Fu *et al*. 2008) and plant yield (Levitt 1980) can also be used as indicators of plant susceptibility to heat stress.

In this study, we used O/P ratio, chlorophyll content, thermal aggregation of leaf proteins and plant yield to assess heat tolerance in two cultivars of spring wheat. The results showed that cultivar Excalibur displayed better ability to tolerate heat stress than cultivar Kukri. Under heat stress conditions, Excalibur

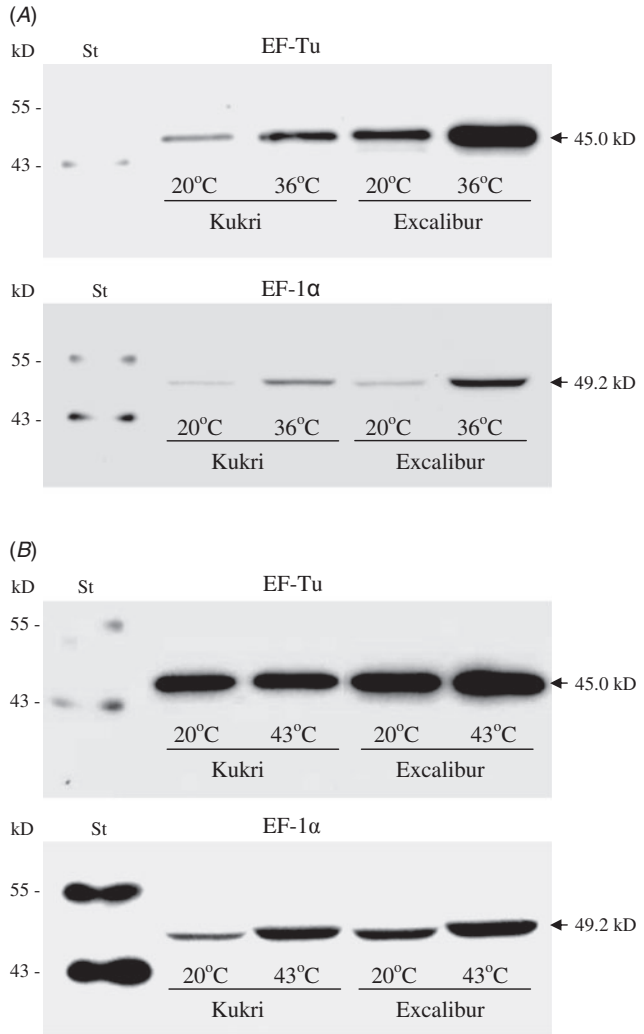

Fig. 4. Immunoblot analysis of leaf total soluble protein extracts from mature (*A*) and young (*B*) plants of spring wheat cultivars Kukri and Excalibur. Mature plants were exposed to 36/30°C, and flag leaf samples were collected after 7 days of heat stress. Young plants (36-day-old) were exposed to 43°C for 24 h, and leaf samples were obtained from the youngest fully expanded leaves immediately after heat stress. Equal amounts of protein (15 μg) were loaded in each lane. Similar results were obtained in duplicate blots. St, protein standards.

showed delayed damage to thylakoid membranes and greater ability to retain chlorophyll than Kukri (Fig. 1). Excalibur also showed better ability to decrease protein aggregation under heat stress than Kukri (Fig. 2). In addition, the yield of Excalibur was less affected by heat treatment than yield of Kukri (Fig. 3). The results on the assessment of heat tolerance in Kukri and Excalibur are consistent with previous reports on genetic variability of heat tolerance in wheat (Wardlaw *et al*. 1980; Blum 1986; Al-Khatib and Paulsen 1990; Ristic *et al*. 2008*a*).

Exposure of mature plants to heat stress (7 days) resulted in increased accumulation of chloroplast protein synthesis elongation factor EF-Tu. The heat-induced accumulation of EF-Tu was evident in both cultivars, but the level of expression differed. The more heat-tolerant cultivar, Excalibur, accumulated EF-Tu to a greater extent than the less heat-tolerant cultivar, Kukri. Similar differential expression of chloroplast EF-Tu has been also observed in winter wheat (Ristic *et al*. 2008*a*) and maize (Momcilovic and Ristic 2004, 2007). A group of winter wheat cultivars with higher tolerance to heat stress displayed greater accumulation of chloroplast EF-Tu under heat stress conditions than a group of winter wheat cultivars with lower tolerance to heat stress (Ristic *et al*. 2008*a*). Likewise, a heat tolerant maize line, ZPBL 1304, accumulated greater amounts of EF-Tu under heat stress than a heat- susceptible line ZPL 389 (Momcilovic and Ristic 2004, 2007).

The heat-induced accumulation of chloroplast EF-Tu in mature plants of Kukri and Excalibur and the higher levels of this protein in the heat-stressed plants of the cultivar that displayed higher tolerance to heat stress (Excalibur) suggest that EF-Tu may play a role in heat tolerance in spring wheat. It is possible that EF-Tu may confer heat tolerance by acting as a molecular chaperone and protecting heat-labile proteins from thermal damage (aggregation). This protein has been shown to display chaperone activity in prokaryotes (Caldas *et al*. 1998), maize (Rao *et al*. 2004), and wheat (Ristic *et al*. 2007*a*), and a recent study demonstrated that heterologous expression of EF-Tu reduces thermal aggregation of leaf proteins in spring wheat following heat stress (Fu *et al*. 2008). Our results on thermal aggregation of leaf protein extracts (Fig. 2) are in agreement with the hypothesis that EF-Tu plays a role in heat tolerance by acting as a molecular chaperone. Furthermore, chloroplast EF-Tu may be involved in heat tolerance through its well characterised function in protein synthesis (Riis *et al*. 1990; Nissen *et al*. 1995). Increased expression of EF-Tu under heat stress may enhance the overall efficiency of protein synthesis and this, in turn, may improve the cell's ability to alleviate the negative effects of heat stress. In addition, EF-Tu may be contributing to heat tolerance through other mechanisms. This protein is involved in several cellular activities, such as the formation of RNA replicase (Blumenthal *et al*. 1972), interaction with adenylate cyclase (Reddy *et al*. 1986), the formation of cytoskeleton-like filament bundles (Beck 1979), catalysis of protein refolding (Richarme 1998) and regulation of transcriptional activation (Young and Bernlohr 1991). It has been suggested that EF-Tu may be contributing to heat tolerance through its involvement in protein refolding (Richarme 1998) and transcriptional activation (Young and Bernlohr 1991). More studies, however, are needed to clarify the mechanism(s) by which EF-Tu may confer heat tolerance.

Exposure to heat stress resulted in increased expression of cytosolic protein elongation factor EF-1α in mature plants of Kukri and Excalibur, and the pattern of EF-1α expression was similar to that of EF-Tu expression. Heat-stressed plants of Excalibur displayed greater accumulation of EF-1α than heat-stressed plants of Kukri. Heat-induced accumulation of EF-1α has been previously reported in fish (Buckley *et al*. 2006) but has not been reported in plants. In rice, Li and Chen (1999) observed that heat shock (37°C) induced accumulation of EF-1α transcripts, but the level of EF-1α protein remained unknown. In wheat, Gallie *et al*. (1998) found that heat shock (20, 45, or 90 min at 41°C) had no effect on the amounts of EF-1α protein in excised leaves of 5-d-old seedlings. Differences in the expression of EF-1α between our study and that by Gallie *et al*. (1998) may be due to differences in experimental conditions and/or plant age. In

our experiments, EF-1α expression was examined in intact flag leaves of mature plants experiencing 7-days heat stress at 36/30°C (day/night), and in the experiments by Gallie *et al*. (1998) EF-1α expression was investigated in excised leaves of 5-day-old seedlings encountering brief (20–90 min) heat shock at 41°C.

The importance of EF-1α overexpression in wheat response to heat stress is unclear. This protein regulates the expression of heat shock proteins (Shamovsky *et al*. 2006) and displays chaperone-like activity (Hotokezaka *et al*. 2002) in mammalian cells. We do not know if EF-1α shows similar activity in plant cells, but because this protein is highly conserved (Riis *et al*. 1990; Bunai *et al*. 2006), it is possible that EF-1α may be an important determinant of heat tolerance in wheat.

The expression of chloroplast EF-Tu in young plants of Kukri and Excalibur under heat-shock was somewhat different from that in mature plants. In young plants, heat shock did not appear to induce a large accumulation of EF-Tu, although a small increase in the level of this protein was noted in heat-shocked plants of Excalibur. Unequal expression of EF-Tu in plants of different age was also observed in *Arabidopsis* (Gallardo *et al*. 2001), maize (Momcilovic and Ristic 2007), and winter wheat (Ristic *et al*. 2008*a*). It is possible that differential expression of EF-Tu in young and mature plants of cultivars Kukri and Excalibur may, in part, be the result of different stress treatments; the young plants were exposed to a short heat-shock (24 h at 43°C) and mature plants experienced prolonged (7 days) exposure to high temperature (36/30°C, day/night). In contrast to EF-Tu expression, the response of EF-1α expression to heat shock in young plants was similar to that in mature plants. The heat-shocked plants of both cultivars displayed a substantial increase in the level of this protein, and the heat-shocked plants of Excalibur appeared to have higher amount of EF-1α than those of Kukri. The significance of EF-1α overexpression in young plants under heat-shock is unclear, but again, for the reasons discussed above, we speculate that this protein may play a role in plant response to heat-shock.

In conclusion, the results of this study showed that heat stress induced accumulation of protein synthesis elongation factors, EF-Tu and EF-1α, in the flag leaf of mature plants of spring wheat cultivars Kukri and Excalibur. The heat-induced accumulation of both elongation factors was greater in Excalibur, the cultivar that showed better tolerance to heat stress. Young plants did not show appreciable accumulation of EF-Tu in response to heat- shock but did, however, display substantial induction of EF-1α, and the overexpression appeared greater in Excalibur than in Kukri. The results support the hypothesis that chloroplast EF-Tu plays a role in heat tolerance in spring wheat. The results also suggest that the cytosolic counterpart of EF-Tu, EF-1α, may be a factor of importance to heat tolerance, and further studies to determine the possible role of this protein in plant response to heat stress are justified.

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